

DATA EVALUATION RECORD

STUDY 4

CHEM 074801

Tribufos

\$162-1

FORMULATION--00--ACTIVE INGREDIENT

STUDY ID 42007204

Stevenson, T.L., W.M. Leimkuehler, and A.E. Mathew. 1991. The Metabolism of Tribufos in Soil Under Aerobic Conditions. Study No. DE042101. Mobay Report No. 100338. Unpublished study performed and submitted by Mobay Corporation, Stilwell, KS.

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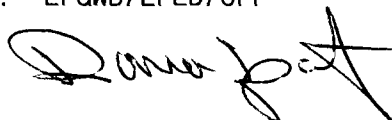
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CONCLUSIONS:Metabolism - Aerobic Soil

1. This study is acceptable and fulfills the aerobic soil metabolism data requirement.
2. Tribufos degraded very slowly in sandy loam soil incubated aerobically in the dark at $25 \pm 1^\circ\text{C}$ for up to 360 days. A 745 day half-life was calculated. The only major degradate identified was 1-butane sulfonic acid, at up to 9.9% of applied radioactivity on day 272.

METHODOLOGY:

Sandy loam soil (58% sand, 27% silt, 15% clay, 3.8% organic matter, pH 6.8, CEC 18 meq/100 g) was sieved (2 mm), dried to 75% of 0.33 bar moisture, and subsamples (100 g dry weight) were transferred to 250-mL Erlenmeyer flasks. The soil was treated at 7 ppm with butyl-labeled [$1\text{-}^{14}\text{C}$]tribufos (radiochemical purity >97.9%, specific activity 20.4 mCi/mMol), dissolved in acetone. The solvent was allowed to evaporate, the soil was mixed, and the flasks were sealed with glass stoppers and parafilm. The flasks were wrapped in aluminum foil to exclude light and incubated at $25 \pm 1^\circ\text{C}$. The flasks were purged with air at 15-day intervals during the course of the study. To trap CO_2 and organic volatiles, air exiting the flasks was vented sequentially through tubes containing dry ice-cooled methanol, methanol, and 1 M NaOH trapping solutions, and then through activated charcoal (Figure 2). The soil moisture was adjusted as necessary. Duplicate flasks were removed for analysis at 0, 3, 7, 14, 29, 59, 181, 272, and 360 days posttreatment.

The soil samples were extracted with acetonitrile using an oscillating shaker; the slurry was separated by vacuum filtration and aliquots of the extract were analyzed by LSC. The extracted soil was then extracted twice with methanol; the slurries were separated and analyzed as described above. Each extract was then concentrated (method not reported). Aliquots of the acetonitrile and combined methanol extracts were analyzed by TLC on silica gel plates developed in hexane:acetone (9:1, v:v). Reference standards of tribufos, dibutyl disulfide, 1-butane sulfonic acid, and methyl-des butylthio tribufos were cochromatographed with the extracts and were visualized by UV (254 nm) or by treatment with sulfuric acid, heating, and charring. Radioactive areas were located by autoradiography and/or a linear radioactivity analyzer. Additional aliquots of the methanol extracts from the 181-, 272-, and 360-day sampling intervals were analyzed by reverse phase TLC on KC18F plates developed in acetonitrile:methanol:0.5 N NaCl (40:40:20, v:v:v). Areas of interest were scraped from the plate, eluted with methanol, concentrated to dryness (method not reported), redissolved in water, and analyzed by HPLC. Aliquots of the concentrated extracts and the eluant of the individual radioactive areas from the TLC plates were analyzed by HPLC using a C-18 column and gradient mobile phase of acetonitrile/tetrahydrofuran (85:15, v:v):0.1% acetic acid. Additional aliquots were analyzed for polar compounds by HPLC using an anion exchange column (RP-18) and a gradient mobile phase of 0.5 N NaCl and water. The eluate from the columns was monitored by a radioactivity detector. Column fractions containing radioactive compounds were concentrated to dryness; the compounds were then redissolved in methanol for analysis by GC/MS.

The extracted soil was then refluxed with 1 N HCl. The slurry was separated by filtration, and the refluxate was concentrated (method not reported). Aliquots of the refluxate were analyzed by HPLC using

an anion exchange column (RP-18) and a gradient mobile phase of 0.5 N NaCl and water. The eluate from the columns was monitored by a radioactivity detector. Eluate fractions containing radioactive compounds were concentrated to dryness (method not reported), and the compounds were redissolved in methanol for analysis by MS. A radioactive compound tentatively identified as 1-butane sulfonic acid isolated from the 181-day soil extract obtained by HPLC was derivatized using N-methyl-N-(t-butyltrimethylsilyl)-trifluoroacetamide, tert-butyltrimethylchlorosilane, and a 2% solution of dioctylphthalate in acetonitrile heated at 70°C. Aliquots of the reaction solution were then analyzed by GC/MS.

Subsamples of the extracted soil were analyzed by LSC following combustion. Aliquots of the trapping solutions were analyzed by LSC; subsamples of the activated charcoal were analyzed by LSC following combustion.

DATA SUMMARY:

Butyl-labeled [1-¹⁴C]tribufos (radiochemical purity >97.9%), at 7 ppm, degraded very slowly in sandy loam soil incubated aerobically in the dark at 25 ± 1°C for up to 360 days. Tribufos was 97.7-100.2% of the applied radioactivity immediately posttreatment, declining to 62.3-66.8% by 360 days (Appendix C). A 745 day half-life was calculated. The degradates identified were

1-butane sulfonic acid,

which was a maximum of 6.9-9.9% of the applied at 272 days posttreatment; and,

methyl-des butylthio tribufos,

which was a maximum of 0.8-1.2% at 181 days. Organic volatiles comprised 2.9-3.9% of the applied radioactivity by the end of the study, and carbon dioxide was 2.9-7.0%. Acid reflux of the extracted soil released an additional 0.4-2.9% of the applied radioactivity which cochromatographed with methyl-des butylthio tribufos. Unextracted radioactivity increased from 0.7-1.5% of the applied immediately posttreatment to 15.4-18.0% at 360 days. The material balances were 91.0-108.9%.

COMMENTS:

1. The registrant-calculated half-life of 745 days is of limited value because the calculations involve extrapolation considerably beyond the experimental time limits of the study. Data are often incapable of accurately predicting trends outside of their range because small differences are magnified and reactions which appear to be linear may, in fact, be curvilinear.

Table 1. Soil Analysis¹

Mobay Soil Number	0383
Textural Analysis (%)	
Sand	58
Silt	27
Clay	15
Class	Sandy Loam
Organic Matter (%) ²	3.8
pH (in 0.01 M CaCl ₂)	6.8
Cation Exchange Capacity ³ (meq./100 g)	18
Particle Density (gm/cc) ⁴	2.6

¹Notebook Reference: 86-R-57 p. 95.²% Organic matter based on total organic carbon * 1.9.³CEC determined by using sodium acetate, pH 8.2.⁴Particle density determined by use of Mobay Ag Chem Report No. 67681.

Table 2. Retention Characteristics for Tribufos and Its Soil Metabolites
In TLC¹ Solvent Systems

<u>Compound</u>	<u>System 1²</u>	<u>TLC Rf</u>	<u>System 3³</u>
		<u>System 2²</u>	
Tribufos (Vial 491 ⁴)	0.25	0.21	0.33
Dibutyl Disulfide (Vial 494)	0.27	0.51	0.28
1-Butane Sulfonic Acid-sodium salt (Vial 495)	-	-	0.87
Methyl-des Butylthio Tribufos (Vial 493)	-	-	0.70

¹TLC solvent systems:

1. Hexane/Acetone (9:1)
2. Hexane/ Ethyl Acetate (85:15)
3. Acetonitrile/Methanol/0.5 N Sodium Chloride (40:40:20)

²Rf values on Whatman Analytical 0.25-mm silica plates

³Rf values on Whatman KC18F 0.25-mm reverse phase TLC

⁴Number in parentheses is Mobay Standard ID number.

Table 3. HPLC Solvent Systems and Retention Times for the Analysis of Tribufos and its Metabolites

Compound	Retention Time (minutes)	
	System 1 ¹	System 2 ²
Tribufos	26	--
Dibutyl Disulfide	28	--
1-Butane Sulfonic Acid	--	21
Methyl-des Butylthio Tribufos	--	5

¹System 1; Column, Econosil C-18, 10 μ , 250 mm x 4.6 mm; Solvent A, ACN/tertrahydrofuran (85:15), Solvent B, water/acetic acid (99.9:0.1); 0 time 50% B, 15 minutes, 20% B, 30 minutes 10% B, 35 minutes 5% B.

²System 2; Column, Alltech anion exchange mixed mode RP-18, 7 μ , 250 x 4.6 mm; Solvent A, 0.5 N NaCl, Solvent B, water; 0 time 100% B, 10 minutes 50% B, 20 minutes 50% B, 30 minutes 0% B, 40 minutes 0% B.

Appendix C

Percent Distribution of Applied Radioactivity in [^{14}C]-Tribufos Treated
Soil Maintained Under Aerobic Conditions for 360 Days

Compound	Day 0		3		7		14		29		59		91		181		272		360	
	REP1	REP2	REP1	REP2	REP1	REP2	REP1	REP2	REP1	REP2	REP1	REP2	REP1	REP2	REP1	REP2	REP1	REP2	REP1	REP2
Tribufos	97.7	100.2	102.5	102.0	98.5	99.5	98.5	101.2	92.8	94.5	86.9	89.2	90.1	91.6	80.8	79.6	72.0	72.3	66.8	62.3
1-Butane Sulfonic Acid	0.0	0.0	1.6	1.8	1.6	1.7	1.7	1.4	4.5	4.8	4.1	3.7	2.0	2.4	5.6	5.1	9.9	6.9	8.3	0.4
Methyl butylthio tribufos	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.8	0.5	0.6	0.0	0.3
Organic Volatile	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.3	0.4	0.2	0.4	0.7	1.2	1.3	1.7	2.4	2.9	3.9
CO	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.1	0.7	0.1	0.7	0.3	0.5	0.6	0.9	1.8	2.9	7.0
Acid Released	0.4	0.4	0.9	0.9	1.0	0.8	0.9	1.0	1.6	1.6	1.7	2.1	2.3	2.5	2.6	2.9	2.5	2.8	2.6	1.7
Bound	1.5	0.7	2.1	2.6	3.0	2.1	3.0	3.7	6.3	6.3	8.4	6.5	8.7	11.4	9.6	11.2	11.1	11.2	18.0	15.4
Total	99.5	101.3	107.2	107.3	104.1	104.2	104.1	107.3	105.5	107.6	102.1	101.8	104.3	108.9	101.6	101.7	98.6	98.1	101.4	91.0
Average	100.4		107.2		104.2		105.7		106.6		102.0		106.6		101.7		98.4		96.2	

¹Values reflect the percent of applied radioactivity.

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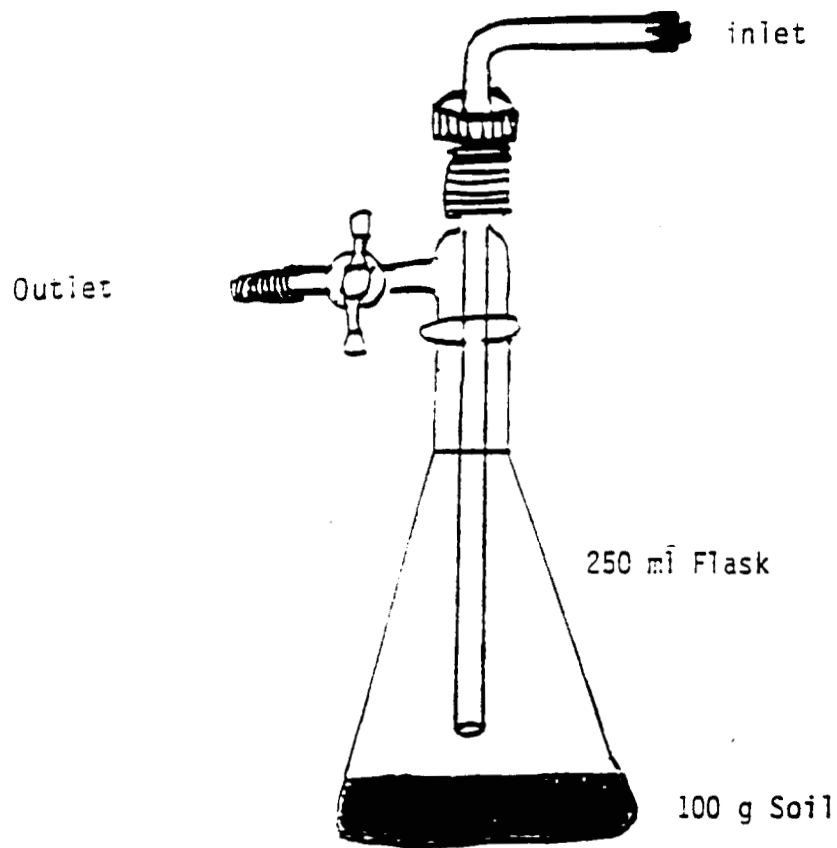


Figure 1. Diagram of the apparatus used to perform the soil metabolism study.

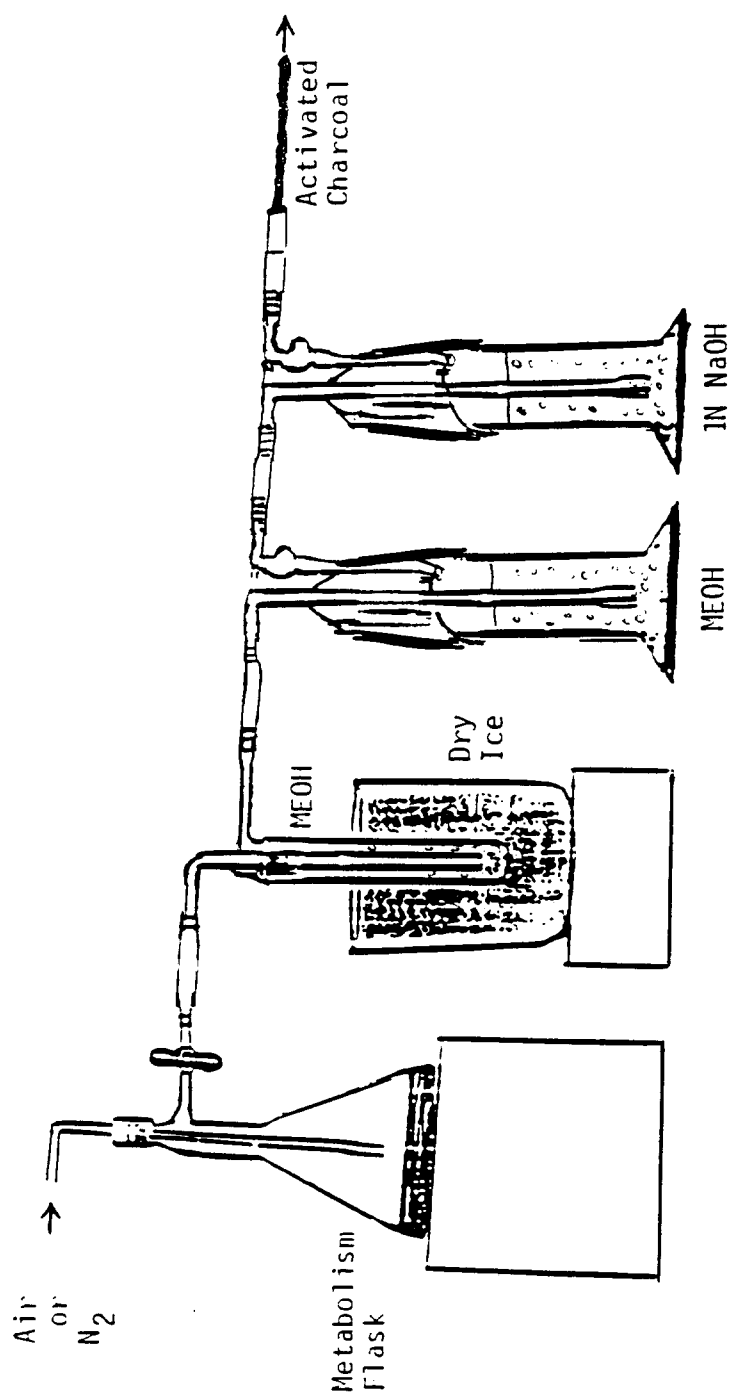


Figure 2. Trapping system used to trap volatile [^{14}C] material.

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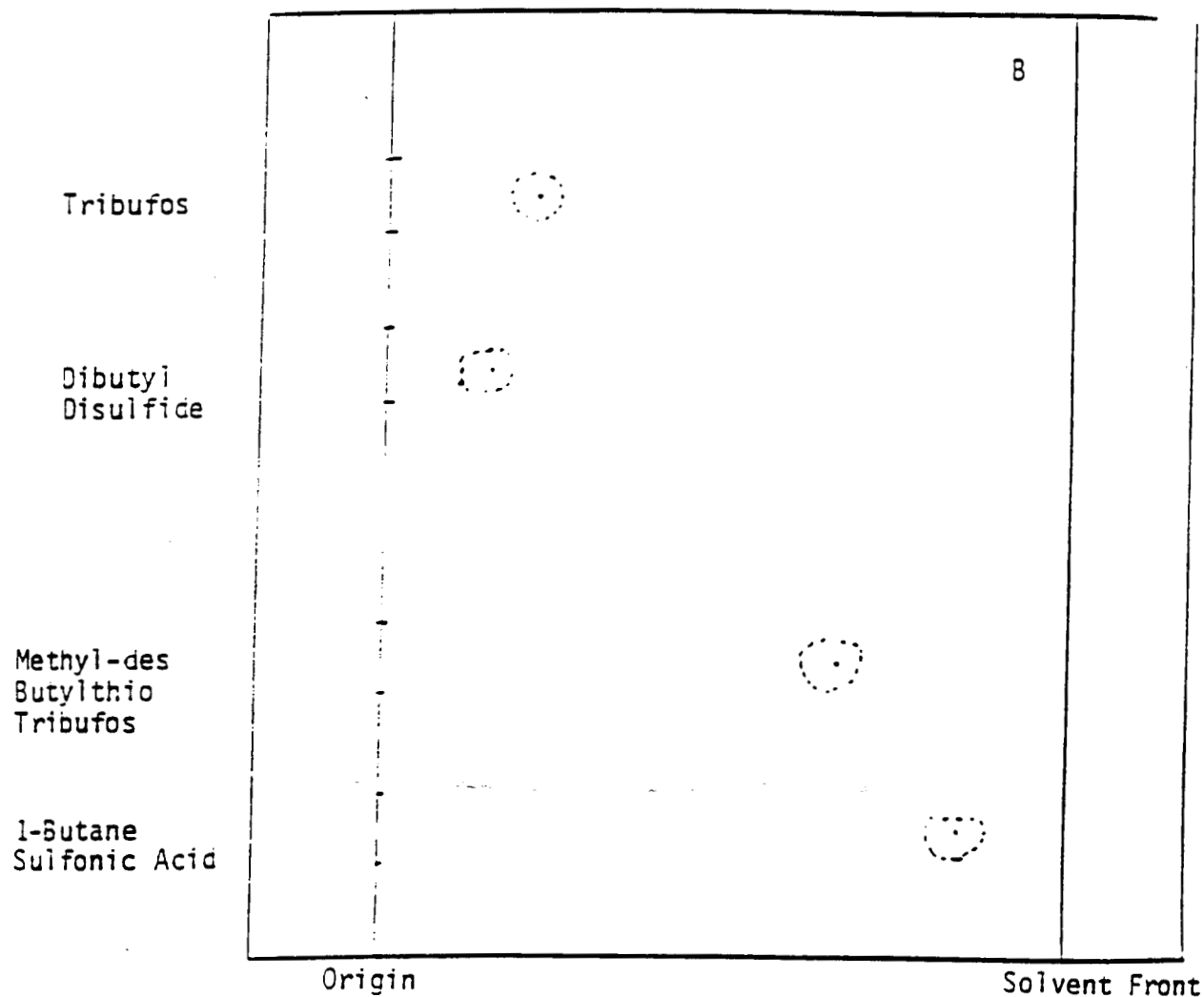
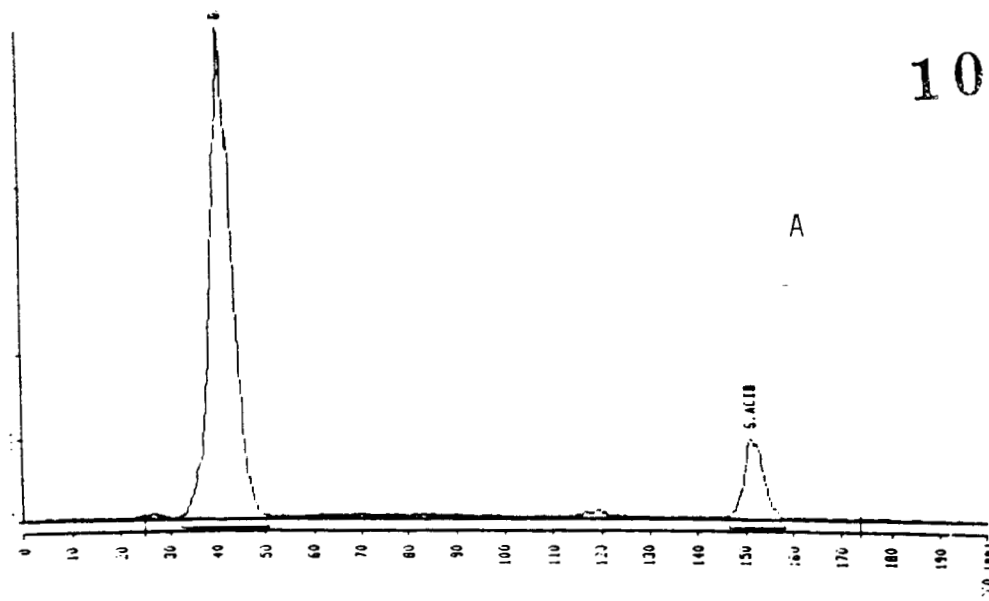


Figure 4. TLC scan (A) of a 360 day methanol extract developed in solvent system 3 on a reverse phase TLC plate. B is a diagram of a plate illustrating the position of the standards.

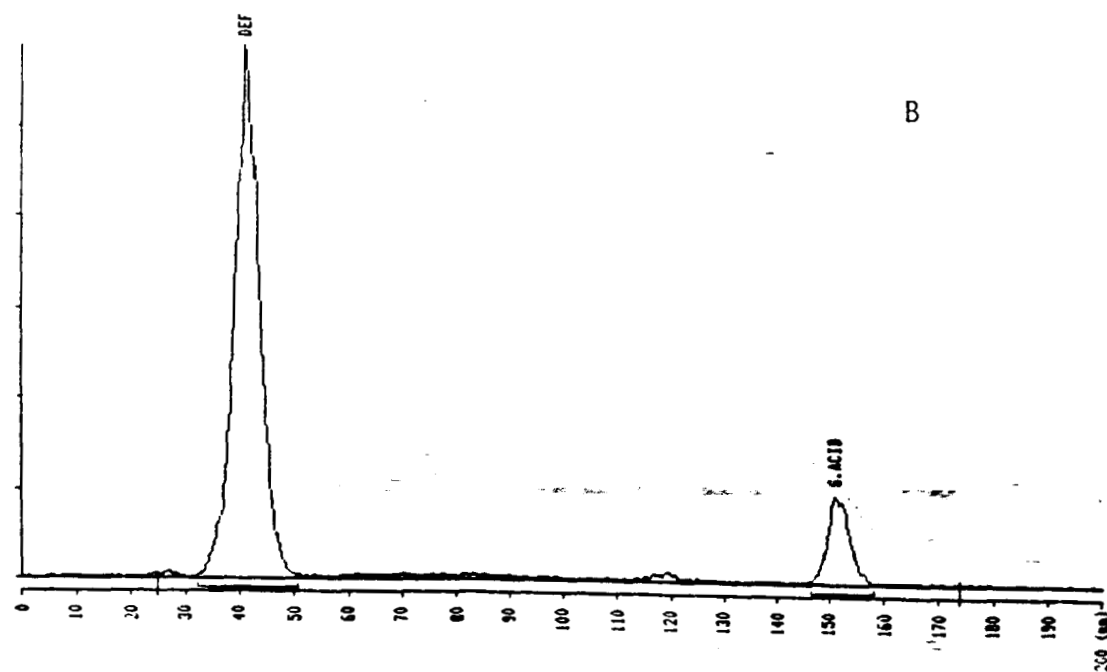
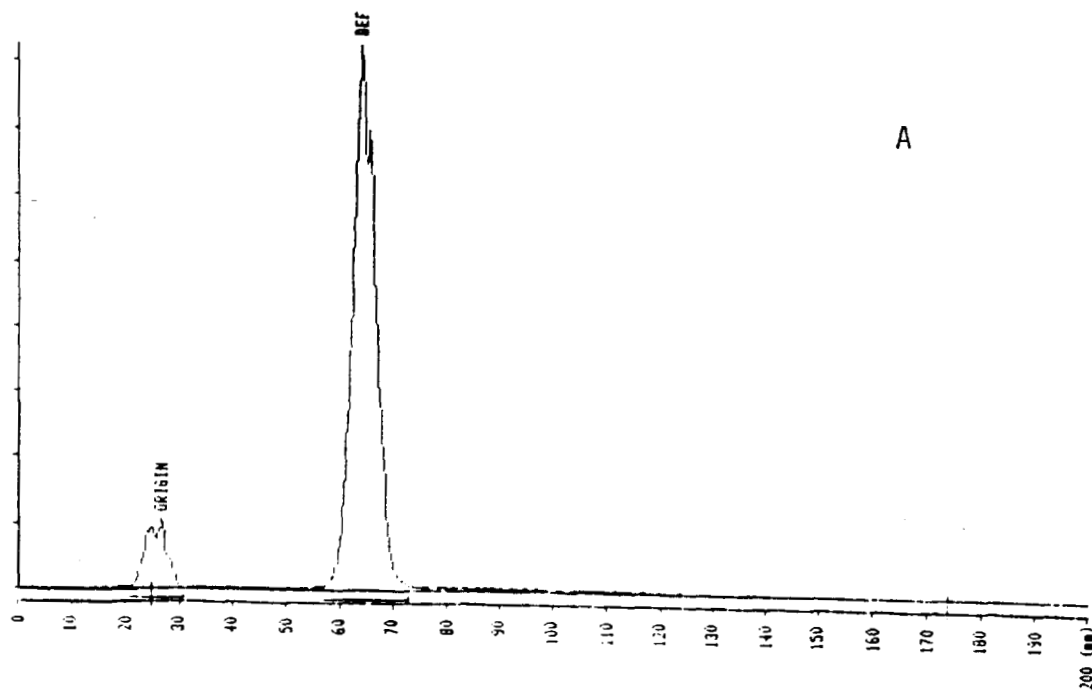


Figure 5. TLC scan of a 360-day methanol extract (A) developed with solvent system 1. B is the same extract developed in solvent system 3.

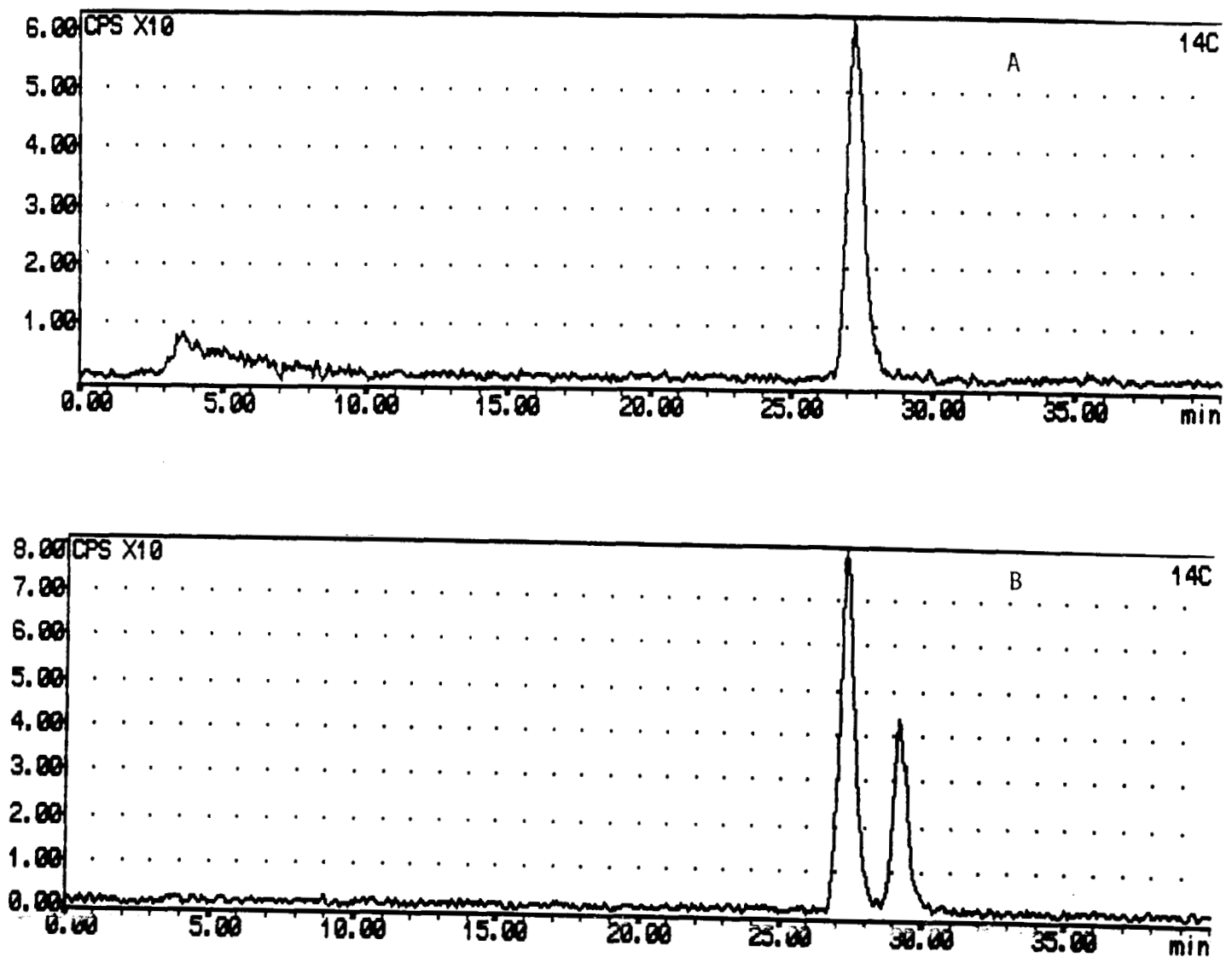


Figure 6. Radiochromatogram of tribufos isolated from soil sampled at 272 days (A) and a radiochromatogram (B) of tribufos and dibutyl disulfide (DBS) ^{14}C standards. HPLC solvent system 1 was used.

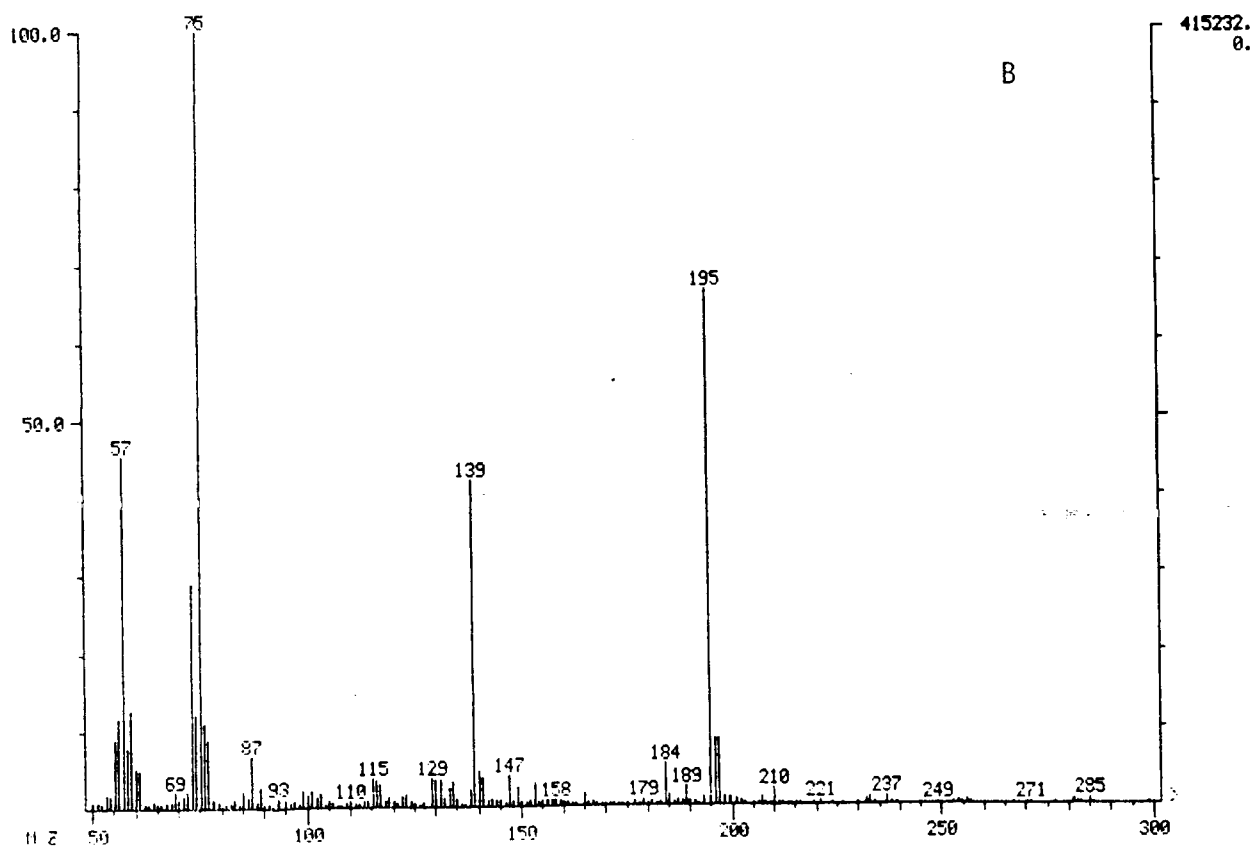
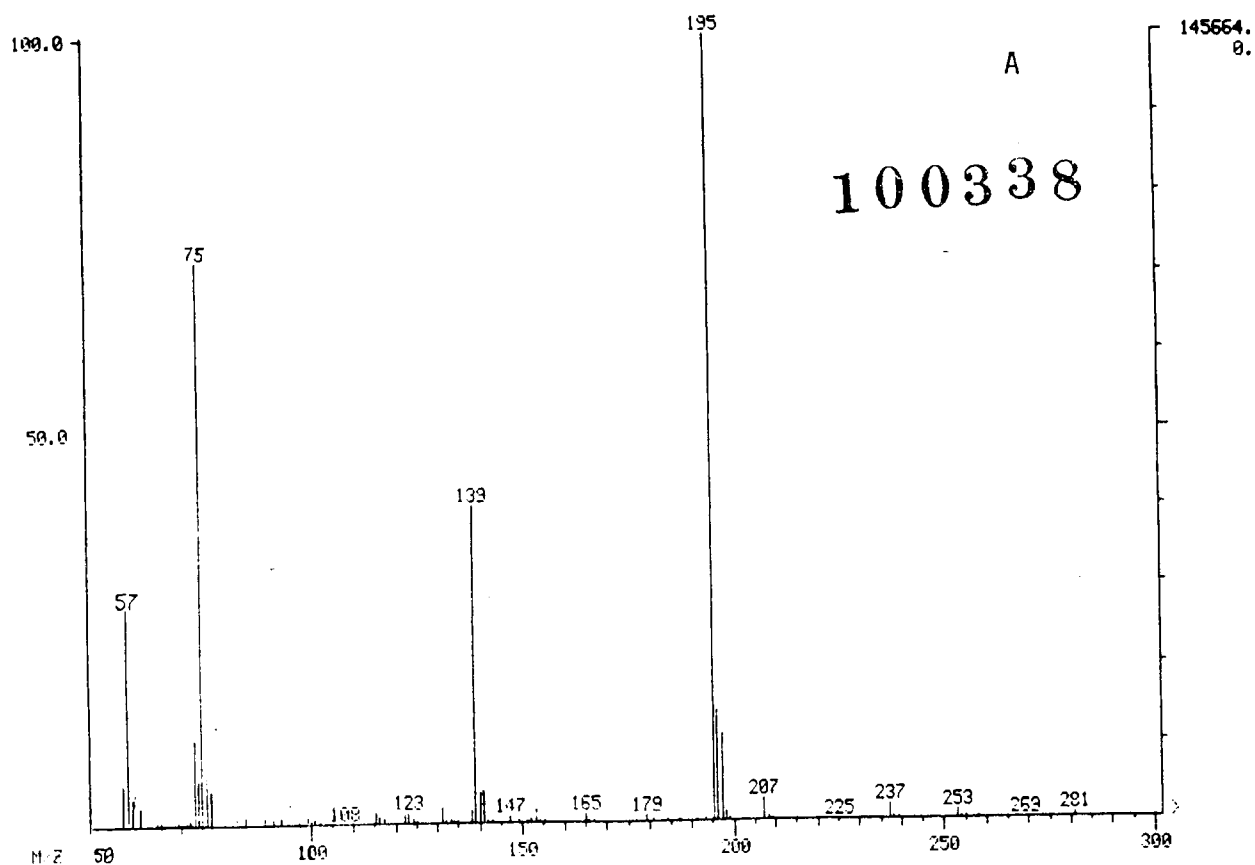


Figure 8. Mass spectrum (A) of the dimethyl-t-butyl silyl ester of 1-butane sulfonic acid. Mass spectrum B is the same derivative of 1-butane sulfonic acid isolated from soil from the 181-day interval. Mass 195 represents loss of a butyl moiety. Mass 139 is loss of the silyl fragment. Masses 75 and 57 are the butyl sulfonate and butyl groups, respectively.

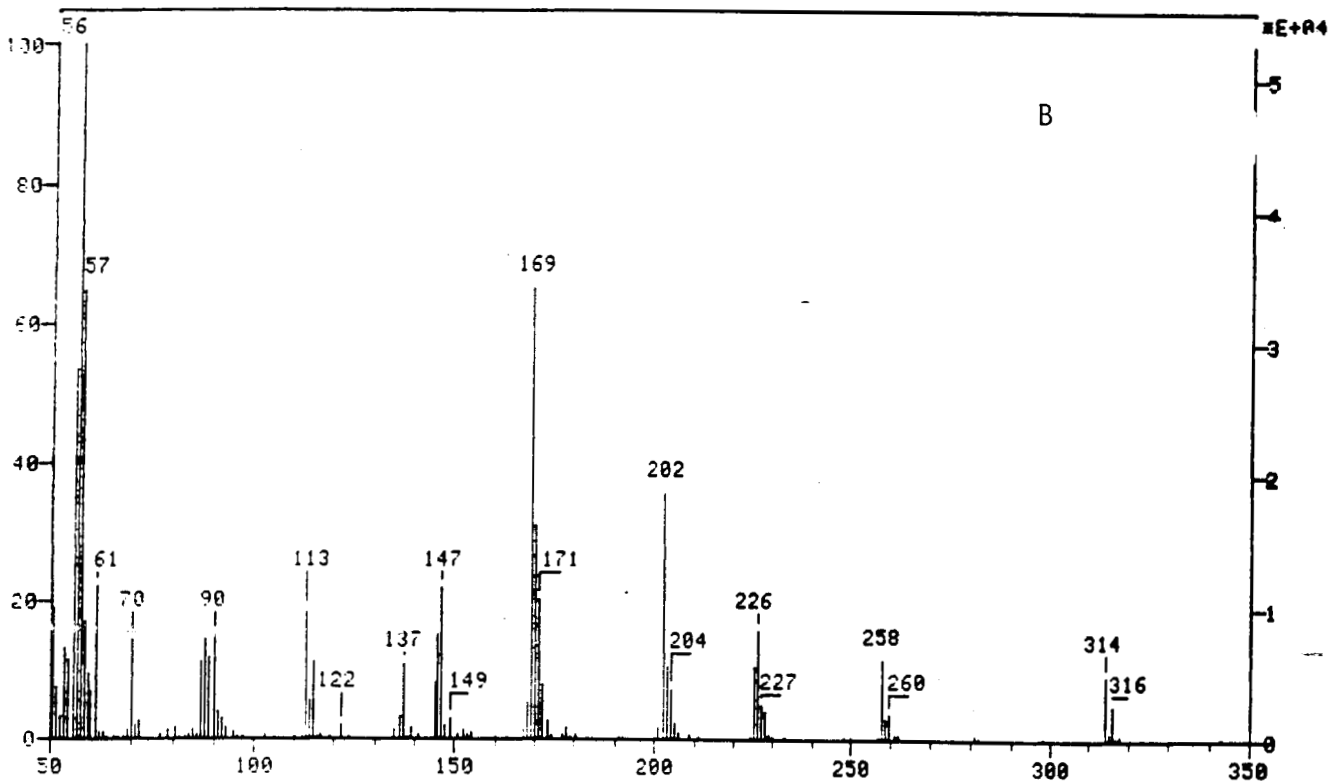
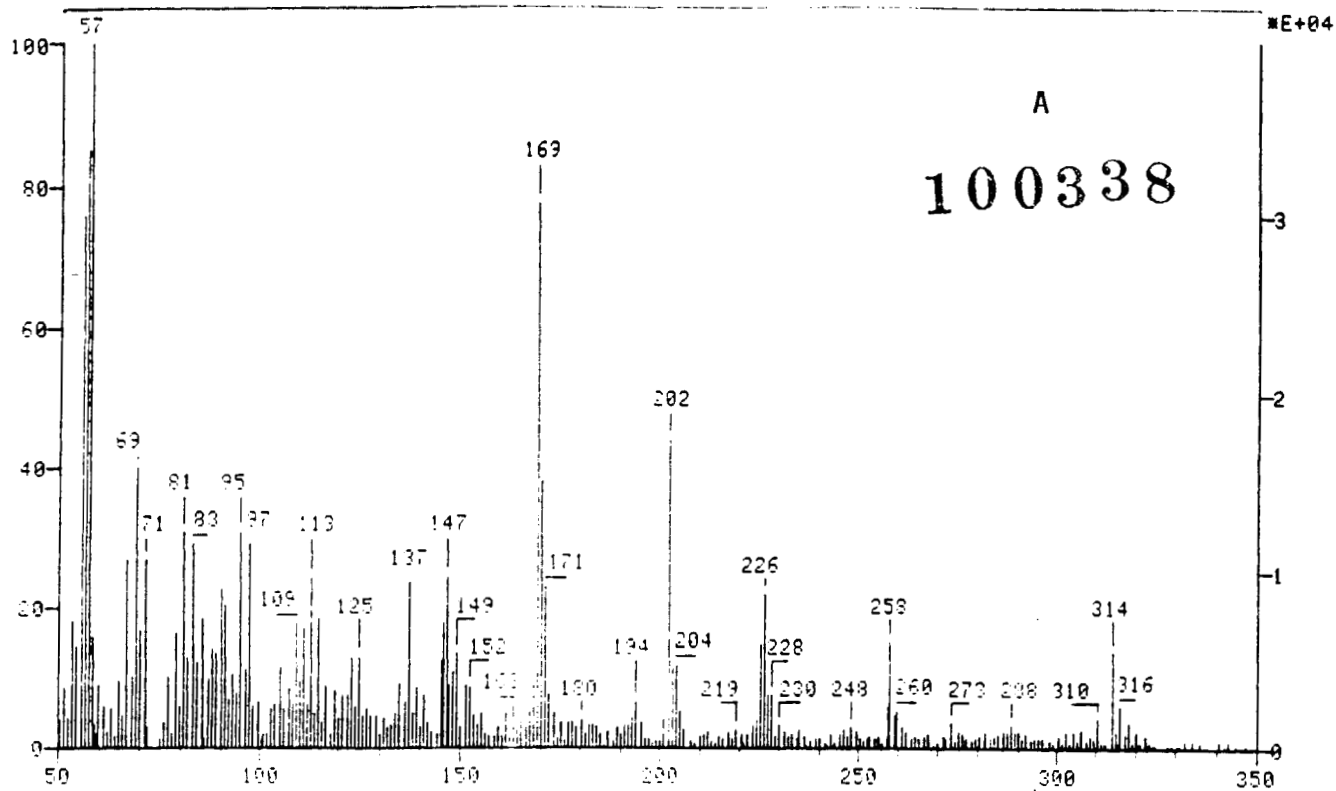


Figure 9. Mass spectrum (A) of tribufos isolated from a 360-day soil extract and mass spectrum (B) of a tribufos standard. Mass 258 represents loss of a butyl group and mass 202 represents loss of two butyl groups.

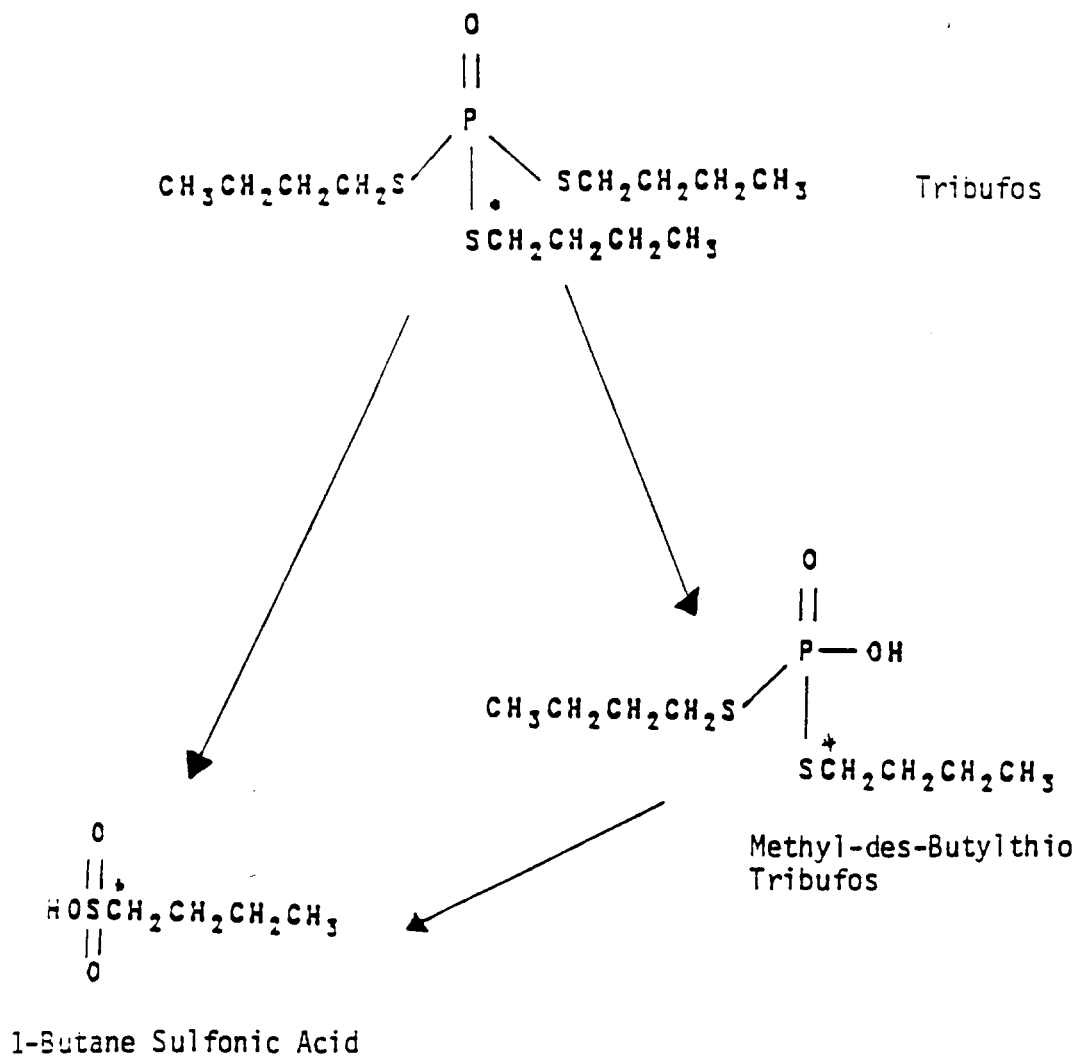


Figure 11. Proposed metabolic pathway for the degradation of tribufos in sandy loam under aerobic conditions.